phrases "a first sequencing reaction product" and "a second sequencing reaction product" in conjunction use of the phrase "two or more short sequencing reaction products." Moreover, claims 2 and 3 are indefinite over the recitation of the phrase "the sequencing reaction product."

Claims 1-5 as amended clarify the actual active steps of a process drawn to identifying a nucleotide sequence of a nucleic acid and references to "sequencing reaction products" have been clarified.

(Note: In what is presumably a typographical error, the office action states that claims 1-**6** are indefinite for having a preamble drawn to "identifying a nucleic acid," however, only claims 1-5 are drawn to a method of identifying a nucleic acid." Claim 6 is an independent claim drawn to "determining a nucleotide sequence.")

The Office Action states that Claims 6-12 are determined to be indefinite for failing to recite a final process step that clearly relates to the preamble and are indefinite over the recitation of the term "the inserts of the library" in claim 6. Claims 6-13 are determined to be indefinite over the recitation of the terms "selected enzyme" and/or "selected portion."

The Office Action describes claims 7 and 9 as indefinite over the terms "the same analysis run" and "the selected restriction enzyme", respectively, as having no antecedent basis. Claims 10-12 are described as indefinite over the recitation of the limitation "the analysis" and states it is unclear how the step of analyzing contributes to "determining the nucleotide sequence."

Claims 6-11 as amended clarify how the step of analyzing contributes to "determining the nucleotide sequence and references to "sequencing reaction products" have been clarified. The relationship of the final process step to the preamble is also clarified. References to "selected portions," "selected enzyme," "the inserts of the library," "the same analysis run," "the selected restriction enzyme" and "the analysis" have also been clarified.

35 USC 103 Obviousness

In sections 7 and 8 the Examiner rejects claim 13 under 35 USC 103(a) as being unpatentable over Kinzler et al. , U.S. Patent No. 5,695,937("Kinzler")

Applicant has canceled claim 13.

Applicant submits that the claims as amended are in condition for allowance and request favorable reconsideration.

Respectfully submitted,

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Analysis may be performed by any means desired. For example, analysis of gel electrophoresis, analysis on a capillary apparatus, or analysis by mass spectrophotometry can be performed.

Also provided is a kit for performing multiplex analysis of sequencing reactions comprising: an enzyme that cuts at least I base downstream of a selected-enzyme recognition site; and a set of oligonucleotide linkers comprising a recognition site for the selected enzyme. For example, the enzyme can be Bpm I, Bsg I, Eco57,or MmeI or a combination thereof The kit can further comprise, for example, a vector io for constructing a library wherein, for example, the vector has an appropriate cloning site for use in the method. The kit can further comprise a component to facilitate the multiplexing of the sequence reaction products, selected according to the analysis method to be used.

Examples

cDNA library construction. Polyadenylated RNA was isolated from 5 x 107 THP1 cells using FastTrack 2.0 (Invitrogen, San Diego, CA). A random oligomer primed cDNA library was constructed from 5 ~Lg of the polyA-selected mRNA using the Copy Kit (Invitrogen). E. coli DNA ligase was removed from the second- strand synthesis reaction to enhance synthesis of products approximately 900 base pairs in length. Next, BpniI linkers (SEQ. ID. NO 1 5'-AATTCGGCTCGAGCTGGAG-3' and SEQ. ID No. 2 5'- CTCCAGCTCGAGCCG-3') were added to the ends of the blunt-ended cDNA fragments using T4 DNA ligase. Following the addition of the linkers, the fragments were phosphorylated (T4 DNA kinase) and size selected using a Chromaspin 400 column (Clontech, Palo Alta, CA). The cloning vector pYesTrp2 (Invitrogen) was digested using the restriction endonuclease EcoRl at 37 'C. The linearized vector was dephosphorylated with shrimp alkaline phosphatase (SAP, Boerhinger Mannheim) prior to gel purification. CDNA inserts and treated, linearized vector DNAs were ligated into the cloning vector and the litigation product was transformed into

1. (Amended)A method of identifying a nucleotide sequence of a nucleic acid comprising:

[performing gel or capillary electrophoresis on a series of two or more short sequencing reaction products loaded sequentially onto the same lanes of a sequencing gel,] loading a first sequencing reaction product [being loaded] at a first loading time into one or more lanes of a sequencing gel; [and]

loading a second short sequencing reaction product [being loaded] onto the same one or more lanes of the sequencing gel as the first short sequencing reaction product at a second loading time, wherein the first loading time and the second loading time are sufficiently temporally separated to separate the first sequencing reaction product from the second sequencing reaction product by electrophoresis; and

performing gel or capillary electrophoresis on the first short sequencing reaction product and on the second short sequencing reaction.

2. (Amended) The method of claim 1, wherein the first sequencing reaction product is produced from a region comprising a SNP (single nucleotide polymorphism).

3. (Amended) The method of claim 1, wherein the first sequencing reaction product is produced from an EST (expressed sequence tag).

4. (Amended) The method of claim 1, wherein the first short sequencing reaction product and second short sequencing reaction [products] product are each about 20 bases or shorter.

5. (Amended) The method of claim 1, wherein the first short sequencing reaction [products are] product is a run off sequencing reaction [products] product.

6. (Amended) A method of determining the nucleotide sequence of a [selected] portion of a nucleic acid comprising:

a) isolating the nucleic acid from a nucleic acid library wherein the library comprises a recognition site of [a selected] an enzyme that cuts at least

1 base downstream of the recognition site, wherein the recognition site is positioned within 1 base of [the inserts] an insert of the library;

b) amplifying the nucleic acid;

c) digesting the amplified nucleic acid with the [selected] enzyme;

d) performing a run-off sequencing reaction utilizing a primer that hybridizes to a region of the amplified fragment at or upstream of the recognition site to form a first sequencing reaction product; [and]

e) loading a first sequencing reaction product at a first loading time into one or more lanes of an electrophoresis sequencing device; and f) performing electrophoresis analysis on [analyzing] the first sequencing reaction product.

7. (Amended) The method of claim 6, further comprising the steps of g) loading a second sequencing reaction product onto the same one or more lanes of the an electrophoresis sequencing device as the first sequencing reaction product at a second loading time, wherein the first loading time and the second loading time are sufficiently temporally separated to separate the first sequencing reaction product from the second sequencing reaction product by electrophoresis; h) and performing electrophoresis analysis on the [wherein a] second sequencing reaction product [is analyzed sequentially on the same analysis run as the first sequencing reaction product].

8. (Amended) The method of claim 6, wherein the [selected] enzyme is a restriction enzyme.

9. (Amended) The method of claim 8, wherein the [selected] restriction enzyme is BpmL

10. (Amended) The method of claim 6, wherein the [analysis] electrophoresis performed is gel electrophoresis.

11. (Amended) The method of claim 6, wherein the [analysis] electrophoresis is performed with a capillary apparatus.

12. (Canceled).

13. (Canceled).

14. (New) A method of determining the nucleotide sequence of a portion of a nucleic acid comprising:

a) isolating the nucleic acid from a nucleic acid library wherein the library comprises a recognition site of an enzyme that cuts at least 1 base downstream of the recognition site, wherein the recognition site is positioned within 1 base of an insert of the library;

b) amplifying the nucleic acid;

c) digesting the amplified nucleic acid with the enzyme;

d) performing a run-off sequencing reaction utilizing a primer that hybridizes to a region of the amplified fragment at or upstream of the recognition site to form a first sequencing reaction product; and

e) performing mass spectrophotometry on the first sequencing reaction product.